## Supplementary materials

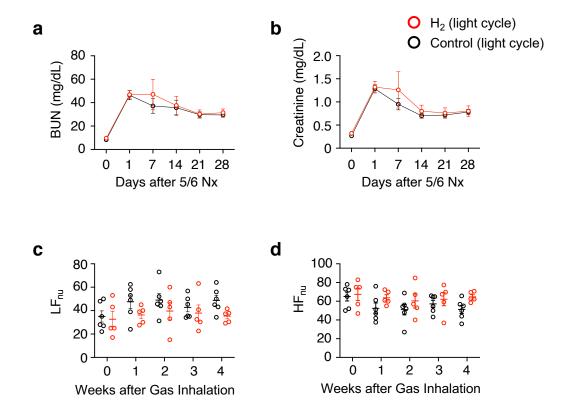
## Daily inhalation of hydrogen gas has a blood pressure–lowering effect in a rat model of hypertension

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а Invasive Blood Pressure Measurement 5/6 Nx Daily Gas Inhalation  $H_2 + 5/6 Nx (N = 3)$ 0 Weeks after Nx Control 5/6 Nx (N = 3) Sham (N = 2)d b C Urine Volume (mL/day) Ccr (mL/min/kgBW) 0.5 0 0.5 0 0.5 0 50 40 30 20 10 0 40 30 20 10 0 2 3 2 3 0 2 3 Weeks after 5/6 Nx Weeks after 5/6 Nx Weeks after 5/6 Nx е g Left Kidney Weight (g) 1.6 1.0 400 Creatinine (mg/dL) Body Weight (g) 8.0 1.2 350 0.6 8.0 300 0.4 0.4 0.2 0 0 0 2 0 2 3 3 Sham Control  $H_2$ Weeks after 5/6 Nx Weeks after 5/6 Nx 5/6 Nx h Diastolic BP (mmHg) Mean BP (mmHg) ‡ 150 150 <del>8-0</del> <del>%</del> 100 100 50 50 Control Control Sham  $H_2$ Sham Control  $H_2$ Sham  $H_2$ 5/6 Nx 5/6 Nx 5/6 Nx

Supplementary Fig. S1. One-hour daily  $H_2$  therapy suppresses blood pressure increase after 5/6 nephrectomy. a) Experimental protocol for measuring the effect of early administration of  $H_2$  on the blood pressure of 5/6 nephrectomised (5/6 Nx) rats.

The groups measured using this protocol were as follows: control gas—treated sham-operated (Sham) rats, control gas—treated 5/6 Nx (control 5/6 Nx) rats, and H<sub>2</sub>-treated (5/6 Nx + H<sub>2</sub>) rats. Gas inhalation treatment was started immediately after respective surgery (day 0). After 4 weeks, a pressure transducer was placed at the right femoral artery and arterial blood pressure was measured under inhaled anaesthesia. **b)** Change in urine volume, **c)** creatinine clearance (Ccr), **d)** blood urea nitrogen (BUN) level, **e)** creatinine level, **f)** left kidney weight, and **g)** body weight change after Nx. **h)**–**j)** Comparison of blood pressure (BP) parameters (systolic BP; diastolic BP; and mean arterial pressure [MAP]) 4 weeks after surgery. Data are expressed as the mean  $\pm$  SE. N = 3 in each group. ANOVA; \* P < 0.05, \*\* P < 0.01. Tukey's multiple comparison test;  $\pm$  P < 0.05,  $\pm$  P < 0.01.



Supplementary Fig. S2. Time course change in renal and autonomic function following 5/6 nephrectomy. Blood pressure and heart rate were recorded at 6 h after the end of gas inhalation treatment. BUN, blood urea nitrogen; HF<sub>nu</sub>, high-frequency power in normalised units; LF<sub>nu</sub>, low-frequency power in normalised units; Nx, nephrectomy. Data are expressed as the mean  $\pm$  SE. N = 5 and N = 6 for H<sub>2</sub> and control group, respectively.